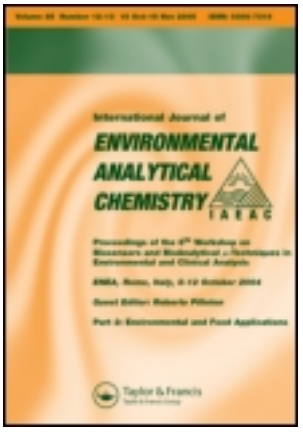

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Determination of vanadium (V) employing a new combined single drop micro-extraction and diffuse reflectance Fourier transform infrared spectroscopy technique

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Determination of vanadium (V) employing a new combined single drop micro-extraction and diffuse reflectance Fourier transform infrared spectroscopy technique†

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The present work deals with a new micro-extraction mode for the selective separation and determination of vanadium in the form of a metaloxy anionic species viz. vanadate (VO_2^+) using N^1-hydroxy-N^1,N^2-diphenylbenzamidine (HDPBA) reagent in to a single drop of dichloromethane and its subsequent and rapid diffuse reflectance Fourier transform infrared spectroscopic (DRS-FTIR) determination on potassium bromide matrix. The vibrational infrared peak at 500 cm^{-1} is selected for the quantitative analysis for the determination. The vanadate combines with the nitrogen and oxygen atoms of N^1-hydroxy-N^1,N^2-diphenylbenzamidine (HDPBA) and forms a selective 1 : 2, V(V) : HDPBA binary complex in acetic acid medium. The chemistry of pure vanadate and that of its HDPBA complex is discussed. The limit of detection (LOD) and the limit of quantification (LOQ) of the method are found to be 40 $\mu g\,L^{-1}$ and 130 $\mu g\,L^{-1}$ V(V), respectively. The precision of the method, in terms of standard deviation and relative standard deviation value, at a level of 10 μg V(V)/5 mL aqueous phase for $n = 10$ is found to be 0.26 μg V(V) and 2.6%, respectively. The relative standard deviation ($n = 6$) for the determination of vanadate (VO_2^+) in some environmental and real human biological fluid samples is observed to be in the range 4.6–7.8%.

Keywords: single drop micro-extraction; DRS-Fourier transform infrared spectroscopy; quantitative determination of V(V); environmental and biological samples

1. Introduction

Vanadium is widely distributed in the earth's crust but in low abundance. Major sources for the emission of vanadium in the environment include combustion of fuel oils, dyeing, ceramics, ink, catalyst and steel manufacturing. Vanadium is reported to be an essential trace element, possessing specific physiological functions for normal cell growth [1,2], but high concentration of vanadium compounds are highly toxic to man and animals [3].

Many separation/preconcentration and determination techniques for the determination of vanadium in biological and water samples have been proposed, including solvent

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extraction [4,5], solid phase extraction (SPE) [6,7], high-performance liquid chromatography (HPLC) [8], cloud point extraction [9], electrothermal vaporization inductively coupled plasma optical emission spectrometry [10] and solid-liquid extraction column [11], hollow-fibre liquid phase micro-extraction [12]. The conventional solvent extraction and co-precipitation however, are laborious techniques and can carry a risk of contamination. Therefore, in recent years, micro-extraction techniques such as dispersive liquid-liquid micro-extraction (DLLME) [13], with capability to perform in-sample and headspace extraction [14–18], continuous flow micro-extraction (CFME) [19,20], solid phase micro-extraction [21], three phase liquid-liquid liquid micro-extraction (LLLME) [22,23], solvent bar micro-extraction [24] and single drop micro-extraction (SDME) [25,26] have been developed based on use of microlitre-level organic solvent. These solvent micro-extraction techniques effectively overcomes the difficulties of solvent losses, large secondary wastes, unsatisfactory enrichment factors, etc. by reducing the amount of organic solvent as well as allowing sample extraction and preconcentration to be done in a single step. The technique is faster and simpler than conventional methods. It is also inexpensive, sensitive and effective for the removal of interfering matrices. Solvent micro-extraction is a form of solvent extraction with phase ratio values higher than 100 [27]. Compared with the conventional solvent extraction, micro-extraction may provide poorer analyte recovery, instead the concentration in the organic phase greatly enhances and thus the detection limit may go down further. In addition, the amount of the used organic solvent is highly reduced and only one step of manipulation is necessary, therefore, problems of contamination and loss of analytes vanishes. Recently, FTIR technique has been employed for quantitative analysis of various chemical species including vanadium [10,28–31].

For the determination of analyte the requirement of minimum sample size in HPLC [8] and graphite furnace atomic adsorption spectrometry (GF-AAS) [32], is usually around 5–20 μL, while in diffuse reflectance Fourier transform infrared spectroscopy (DRS-FTIR) it is around 3–10 μL as reported earlier [25,33–36].

In the present work, a combined single drop micro extraction (SDME), with the use of N^1-hydroxy-N^1,N^2-diphenylbenzamidine (HDPBA), and diffuse reflectance Fourier transform infrared spectroscopy (DRS-FTIR) technique has been developed for the first time for determination of vanadium in environmental and biological samples.

2. Experimental

2.1 *Apparatus and reagents*

All spectral scans in the region 4000–400 cm^{-1} were made employing a diffuse reflectance Fourier transform infrared spectrometer (DRS–FTIR) equipped with an L-alanine doped deuterated triglycine sulfate (DTGS) detector (model-FTIR 8400S, Shimadzu Corporation Analytical and Measuring Instruments Division, Kyoto, Japan). A single beam Systronics spectrophotometer (model-104, Mumbai, India) was used for UV-VIS characterization of the vanadium complex. For homogenous stirring of reaction mixture 5 MLH magnetic stirrer (Remi Equipments Pvt, Mumbai, India) was used.

All reagents and materials used were of analytical grade. Vanadium(V) stock solution of 10 mg L^{-1} was prepared by dissolving a suitable amount of AR grade ammonium metavanadate (Merck KGaA 64271 Darmstadt, Germany) in 100 mL ultra pure water. The solution was standardised spectrophotometrically by 1,5-diphenylcarbazide method [37]. Appropriately diluted solutions of the above standard vanadium(V) solution

were used for further work. Potassium bromide (KBr) used in this analysis was of infrared spectrometric grade, Merck KGaA 64271 Darmstadt, Germany. A 5.0 mol L^{-1} acetic acid was employed for adjusting the acidity of aqueous phase. N^1-Hydroxy-N^1,N^2-diphenyl-benzamidine (HDPBA) was synthesised as according to the descriptions made elsewhere [38] and its solution in dichloromethane (Merck, AR grade) (0.1% w/v or 3.5×10^{-3} mol L^{-1}) was used for extraction and micro-extraction purposes.

2.2 *Procedure for single drop micro-extraction (SDME) of vanadium(V)*

An 8-mL vial (Kimble Glass, Vineland, NJ, USA) with a stirring bar (8 mm × 1.5 mm, Fisher) was placed on a magnetic stirrer. A portion of standard solution containing 10 μg of vanadium(V) was placed in the vial and the acidity of the final 5 mL aqueous phase was adjusted to 0.5–4.0 mol L^{-1} with acetic acid. The vial was sealed with a poly tetrafluoroethylene (PTFE)-coated silicon septum. A 10-μL micro syringe (Gastight, Hamilton, Reno, Nevada, USA) filled with 5 μL of dichloromethane, dissolving 3.5×10^{-3} mol L^{-1} HDPBA in it, was injected into the acidified test solution. The needle tip was immersed into the solution and fixed about 1 cm below the surface of the sample liquid. Then the 5 μL of the above extractant was squeezed out of the needle and kept suspended at the needle tip. The solution was stirred at 400 rpm for 7 min. After the extraction is complete, the drop was retracted back in the micro syringe, and the needle was removed carefully from the sample vial. The needle was wiped with a tissue paper to remove any possible water contamination.

2.3 *Procedure for DRS-FTIR determination of vanadium(V)*

The above extracted micro drop of dichloromethane containing V(V)-HDPBA complex was delivered through micro syringe tip at the centre of the sample cup (dia 4 mm) completely filled with 15 mg finely ground KBr. Then the sample cup filled with KBr matrix was dried in an oven at a temperature around 60°C for 2 min in order to evaporate the solvent. The sample cup was taken out and analysed by the DRS-FTIR for V(V) content against a reagent blank prepared under the similar condition without introducing V(V) in the system. The optimum conditions set for DRS-FTIR analysis of the samples are shown in Table 1.

2.4 *Procedure for preparation of water samples*

A 5 mL portion of industrial waste water sample collected from contaminated sites were filtered employing the usual gravitation filtration technique through a 0.45 mm cellulose acetate membrane filter, after filtration, was directly taken for analysis by the recommended SDME-DRS-FTIR method. However, preconcentration by volume reduction (5–50 folds depending on actual concentration) step was necessary for river, ground, rain and tap water samples.

2.5 *Procedure for preparation of biological samples*

A propene intravenous cannula was used for sampling of blood and cerebrospinal fluid [39]. After discarding the first 2 mL of blood due to possible contamination, the next

Table 1. Instrumental specifications set for the DRS-FTIR analysis of samples for vanadate.

Particulars	Description
Instrument	Fourier Transform Infrared Spectroscopy, 8400S Shimadzu Corporation, Kyoto Japan
Technique	Diffuse reflectance Infrared Fourier Transform Spectroscopy
Software	IR Solution
Sample volume	About 5 microliter
Sample form	Liquid
Appodisation function	Happ-Ganzel
Resolution	$4\,cm^{-1}$
No. of scanning	35
Measurement mode	Absorption
Spectral range (peak identification)	$1520\text{-}450\,cm^{-1}$
Spectral range (quantitative analysis)[a]	$550\text{–}450\,cm^{-1}$
Beam	Internal
Detector	L-alanine-doped deuterated triglycine sulfate
Mirror speed	$2.8\,mm\,s^{-1}$

[a]Baseline correction range for all analysis.

2 mL was withdrawn from a vein in the forearm using a standard plastic Venflon cannula (Viggo AB, Sweden) and the sample was transferred to an acid-washed polypropylene centrifuge tube. After clotting and centrifuging, the serum sample was transferred to an acid-washed 2 mL capped sample vial and preserved at 4–10°C. A 0.2–0.5 mL serum was separated and diluted with 1% v/v HCl acid in a volume ratio of 1 : 4. A 0.5–1.0 mL cerebrospinal fluid from the spine was sampled and the sample was treated similarly with nitric acid. Vanadium could then be determined in human serum without ashes and digestion, by using DRS-FTIR in KBr matrix after SDME process with use of no chemical modifiers, unlike the other methods [40].

A 5 mL urine sample was poured into acid washed 25 mL polyethylene container and to this 0.5 mL of AR grade concentrated hydrochloric acid was added. A 5 mL portion of it was taken directly for SDME extraction and DRS-FTIR determination using KBr matrix as described earlier.

3. Results and discussion

In the present work, SDME combined with DRS-FTIR was developed for the first time. The V(V) in the form of a IR active multiatomic cationic species viz. VO_2^+ was selected as an example to observe the possibility of this combination.

3.1 *Single drop micro-extraction (SDME) of vanadium(V)*

While performing the conventional liquid-liquid solvent extraction (LLSE), which formed the basis of SDME process, it was seen that an acidity range of $0.5\text{–}4.0\,mol\,L^{-1}$ acetic acid in a 10 mL aqueous phase produced constant and maximum extraction (>99%), in terms of absorbance at λ_{max} 560 nm, of V(V) with HDPBA in 5 mL

dichloromethane in only 1 min equilibration time. Strong acids, such as hydrochloric, sulphuric and nitric acid showed overall a very narrow pH range, 1.2–4.4, for quantitative extraction.

The stoichiometry of the complex was determined by the curve-fitting method [41]. The molar ratio of metal to HDPBA was determined by plotting the logarithm of the distribution ratio of the metal $\{\log[A_{eq}/(A_{max} - A_{eq})]\}$ (where A_{max} = maximum absorbance of the complex at λ_{max} at optimum reagent concentrations and A_{eq} = absorbance of the VO_2^+ complex at equilibrium concentration, obtained by equilibration with different known concentrations of HDPBA *versus* the logarithm of various known concentrations of HDPBA in dichloromethane. Thus, the studies on conventional liquid-liquid extraction have shown that a violet coloured 1 : 2, V(V) : HDPBA complex was formed in dichloromethane).

Thus, from the result it could be expected that one mole of vanadate ion (VO_2^+) is attached with 2 moles of staggered N^1-hydroxy-N^1,N^2-diphenylbenzamidine (HDPBA) through two coordinate V–N^2 bonds and two covalent V–O bonds to form a binary VO_2^+-HDPBA complex in acetic acid medium. The formation mechanism of the binary VO_2^+-HDPBA complex, based on above studies, could be represented as per the following equations:

$$NH_4VO_3 + CH_3COOH + 2HDPBA \leftrightarrow [H\{VO_2(DPBA)_2\}]_o + NH_4COOCH_3 + H_2O$$

or for simplicity reaction:

$$VO_3^- + H^+ + 2HDPBA \leftrightarrow [H\{VO_2(DPBA)_2\}]_o + H_2O$$

Here the subscript 'o' denotes to the organic phase.

Further, in the micro drop, the formation of above complex was confirmed by the appearance of violet colour. Of the various tested polar and non-polar organic solvents tested, chloroform and dichloromethane were able to extract complex with maximum colour intensity. However, since dichloromethane is less toxic it was selected for all extraction purpose.

Compared to LLE, the SDME extraction of vanadium shows the relatively narrow range of HDPBA concentration that is suitable for quantitative analysis. However, the upper limit of the concentration range of HDPBA required for maximum enrichment of vanadium complex in dichloromethane droplet remained intact at an optimum micro-drop volume and reaction time of 5 μL and 7 min, respectively. The maximum and steady absorbance intensity of the V(V)-HDPBA impregnated KBr substrate was seen when vanadate was extracted with a single drop of dichloromethane with 0.004–0.01 mol L^{-1} HDPBA in it.

3.1.1 *Optimisation of drop and sample volume*

In SDME the volume of the organic drop does not remain constant during the extraction because no solvent is completely immiscible with water. However, a slight decrease in drop volume is acceptable because it will occur to the same extent for standard and the sample [42]. The effect of microdrop volume on the analytical signal of 10 μg per 5 mL of vanadate reacted with HDPBA in dichloromethane was determined. A test volume of 5 mL aqueous phase containing 10 μg vanadate was reacted with HDPBA in dichloromethane and extracted with 5–7 μL drop volume of the solvent. The results

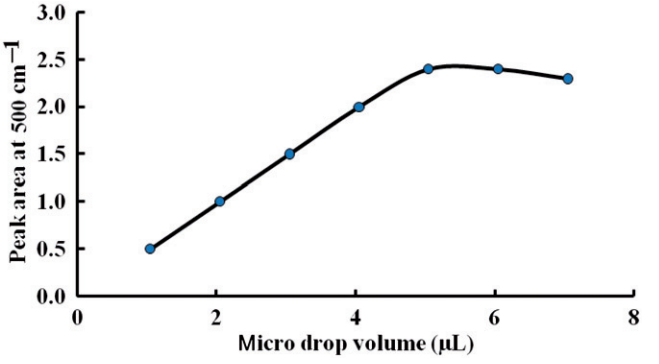

Figure 1. Effect of micro-drop volume on the absorbance of VO_2^+-HDPBA complex. Condition: analyte, 10 μg/5 mL; extraction time, 7 min; stirring rate, 400 rpm.

indicated that increasing the microdrop volume enhanced the extraction efficiency (Figure 1). Drop volume of 5 μL was used in subsequent experiments.

The effect of dilution of the aqueous phase was tested and it was observed that the signal intensity of the VO_2^+-HDPBA complex in organic microdrop at 500 cm^{-1} goes on decreasing steadily with the increase in sample volume, containing fixed overall amount of V(V), from 5 mL to 10 mL at the recommended experimental conditions. This could be attributed to the lowering in extraction efficiency of the systems due to lesser possibilities of phase-interaction up on enhanced dilution. However, this loss could be compensated by increasing the reaction time to 20 min. Hence, a 5 mL sample volume was chosen for all further work.

3.1.2 *Optimisation of stirring rate and reaction time*

Like SPME, LPME is a process dependent on equilibrium [43]. The amount of analyte extracted at a given time is dependent on the mass transfer of analyte from aqueous samples to the organic solvent drop. This procedure requires a considerable period of time for equilibrium. Agitation of the sample reduces time to reach thermodynamic equilibrium and increase extraction efficiencies. To evaluate the maximum extraction efficiency variation in stirring rate and reaction time was done. Water samples (spiked at 10 μg per 5.0 mL with analytes) were extracted with 5 μL of 0.1% w/v HDPBA in dichloromethane at different time intervals with varying stirring rates (100 to 400 rpm). Stirring above 400 rpm were not evaluated because they destabilised the drop. The results typically shown in Figure 2 evidently indicated that at a stirring rate of 100 rpm, it took a longer time to attain the equilibrium, and the extraction was also less. At 400 rpm, a maximum amount of analyte was extracted with the fastest attainment of equilibrium. Therefore, a stirring rate of 400 rpm was fixed for further micro-extractions.

As can be observed in Figure 2, the relative peak area of the VO_2^+-HDPBA complex increased with extraction time up to 5 min. At the 7 min time point, the amount extracted reaches the largest value, and then the value keeps constant. Therefore, in all subsequent optimized experiments, 7 min extraction time was used.

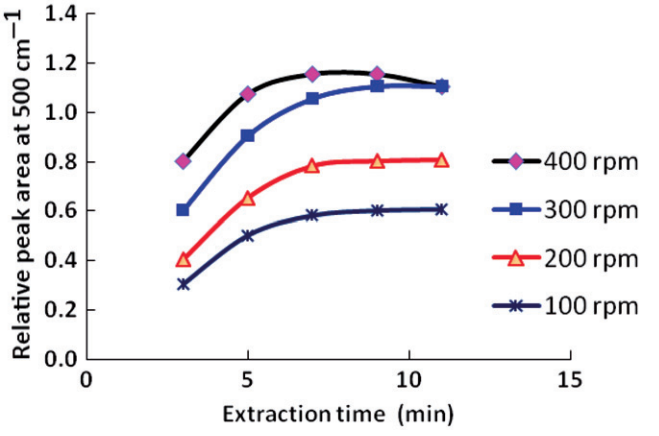

Figure 2. Effect of stirring rate and reaction time on the absorbance of VO_2^+-HDPBA complex. Condition: analyte, 10 µg/5 mL; single drop volume, 5 µL.

3.1.3 *Effect of salting out agents*

The effect of ionic strength of the electrolytes in sample solution on the extraction efficiency of vanadium was evaluated by introducing different concentrations of electrolytes viz. KCl, NH_4Cl and K_2SO_4 in the aqueous phase of the SDME process. It was observed that the extraction was unaffected by the presence of 3 M KCl, 2.5 M NH_4Cl and 3.6 M K_2SO_4 in aqueous phase. Further increase in electrolyte concentration decreased the extraction efficiency, which could be due to the increase in viscosity of the aqueous phase and decrease in mass transfer from the solution into the organic micro-drop.

3.1.4 *Enrichment factor*

To evaluate the enrichment factor in the SDME process, a comparison was made for the sensitivities (slopes) of the calibration curves obtained for the presently reported pre-concentration method and for the method without pre-concentration, i.e. a calibration curve prepared by directly taking portions of 5 µL organic extract of VO_2^+-HDPBA complex, obtained by LLSE, from the bulk of aqueous phase (5 mL) containing different known concentrations of vanadium employing DRS-FTIR analysis at 500 cm^{-1}. An average calibration curve slope ratio of 1 : 28 (for 6 data points each) was obtained for the two methods. Thus, it indicated an enrichment factor of 28 in SDME process.

3.2 *DRS-FTIR determination of vanadate*

3.2.1 *Detection of qualitative vibrational (infrared) peaks for vanadate*

The descriptions on characteristic IR absorption bands for vanadate as found in the literature [44] were used for the interpretation of the FTIR spectra of vanadate functional group in the present work. All the characteristics IR absorption bands for vanadate are checked by employing standard samples. This paper qualitatively identifies the presence of vanadate species by the study of spectra of its pure salt or compound. The presences of

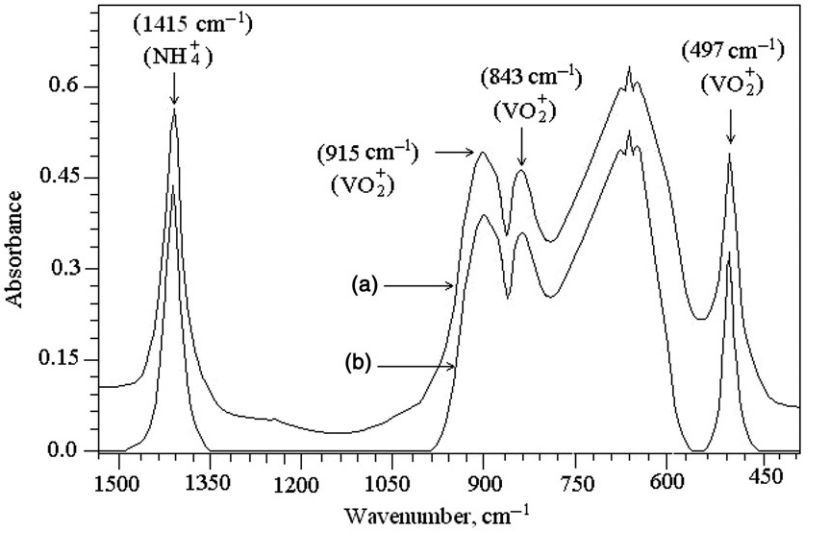

Figure 3. Real DRS-FTIR spectra of VO_2^+ in its purest form in KBr matrix before (a) and after base line correction (b).

vanadate are commonly reported to be related to strong absorption bands at 915, 843 and 497 cm^{-1}. In the present work, the spectral study of the solutions prepared using NH_4VO_3 salt shows 4 strong (broad and sharp) absorption bands at 1415, 915, 843 and 497 cm^{-1} which were attributed to bending (υ_4) vibration for NH_4^+ ion, and asymmetric stretching (υ_3), symmetric stretching (υ_1) and bending (υ_2) vibrations, respectively for VO_2^+ ion.

3.2.2 *Comparison of spectral characteristics of pure VO_2^+ ion and VO_2^+-HDPBA complex*

The spectral peaks obtained for pure vanadate ion, taken in the form of ammonium metavanadate, was compared with that obtained for VO_2^+ -HDPBA complex in SDME, in order to see whether both provide the similar spectral characteristics. Figures 3 and 4a show the spectra scanned for vanadate before and after extraction with HDPBA i.e. VO_2^+ in its purest form and in complexed form, respectively. It was observed that there was only a slight shift (2–5 cm^{-1}) in the position of spectral peaks produced due to both the species, apart from the few those produced due to functional groups present in the HDPBA. This small change in the spectral peak positions may be attributed to the coordination of VO_2^+ with the organic ligand HDPBA. The three corresponding peaks at 915, 843 and 497 cm^{-1} for the pure vanadate were found at 920, 841 and 500 cm^{-1} respectively for the VO_2^+-HDPBA complex. This probably indicates that even after coordinating with the organic ligand, HDPBA, VO_2^+ does not lose its structure and symmetry identity rigorously and it appears as if V–O bonds are as free to oscillate in the complexed form as in the pure form. Thus, the similar appearance of spectral peak positions formed the criteria for identification of the VO_2^+ ion in single drop micro-extract.

3.2.3 *Advantage of complexation of V(V) in single drop micro-extraction*

The present work has been established by performing IR spectral scans of standard VO_2^+ using NH_4VO_3, the other vanadates like $Na_4V_2O_7 \cdot xH_2O$, $Na_3VO_4 \cdot 10H_2O$, $Ca(VO_4)_2$,

$Pb_3(VO)_2$, $FeVO_4 \cdot 2H_2O$ and Ag_3VO_4 etc. may also present in a complex environmental matrix in solid or liquid states along with NH_4VO_3. Since in solid crystalline form the various vibrational modes of VO_2^+ in all above complexes generate absorption maxima with a substantial drift around $\pm 10\,cm^{-1}$, it may possibly be difficult to quantitatively determine the total V(V) in real samples using the spectral peaks of NH_4VO_3 alone as the standard, due to possible overlapping of maxima for different complexes of NH_4VO_3. However, this drift value for position of absorption maxima in liquids is though relatively less due to decreased atomic interaction but cannot be ignored. Thus, the pure spectra of VO_2^+ when used in the form NH_4VO_3 alone cannot be used for quantification of V(V) in real samples. The problem of possible spectral overlapping due to different such vanadate species could be solved by isolating the vanadate ions in solutions through complexing them with an organic ligand such as HDPBA into a separate immiscible phase. Thus, single drop micro extraction of V(V) serves two important aspects, first to eliminate any possible interference due to the presence of associated metal ions in real samples and secondly to remove the possible drift and overlapping of spectra at maxima due to the possible presence of different vanadate species in real samples.

3.2.4 *Quantitative and analytical infrared peak selection for vanadate determination*

Figure 3(a) shows the IR absorption spectra of vanadate in the region 1100–400 cm^{-1}. Although the IR peak observed at 497 cm^{-1} is relatively less strong at high vanadate concentration compared to the peaks at 915 and 843 cm^{-1} but due to consistency in spectral position and its quantitative behaviour the peak at 497 cm^{-1} for bending (υ_2) vibration was chosen for the quantitative determination of vanadate in the pure compound. However, due to a small shift in the above peak position after complexation with HDPBA, a shifted peak at 500 cm^{-1} was used in the real samples analysis. Also, the spectral interferences due to the presence of other possible ions in real samples in regions other than at 500 cm^{-1} were seen. A two-point baseline correction was performed between 550 and 450 cm^{-1} in all cases for the observation of the quantitative spectral peaks due to VO_2^+-HDPBA complex.

3.2.5 *The Kubelka Munk spectrum and calibration curve*

The following equation executes the Kubelka Munk conversion for a spectrum measured by diffuse reflection method:

$$f(\mathrm{R}) = \frac{(1-\mathrm{R})^2}{2\mathrm{R}} = \frac{k}{s}$$

Here k = molecular extinction coefficient; s = scattering coefficient; R = reflectance (power spectrum of sample/power spectrum of dilution material, KBr).

The Kubelka Munk conversion uses the formula above to invert a reflectance spectrum measured by the diffuse reflection method into a quasi-quantitative spectrum that correlates with the concentration of the sample. The calibration curve method executes the quantification of an unknown sample by acquiring a regression equation which represents the relationship between the peak intensity (peak height or absorbance) or peak area in absorbance mode of target ion and concentration from spectra of standard samples whose concentrations are already known. The calibration curve was prepared by employing least squares method for most probable straight-line fit of the $x - y$ (concentration-absorbance)

data using the equation $y = mx + c$; y is usually the measured dependent variable (absorbance/peak area) plotted as a function of changing x (concentration).

Thus, the calibration curves for peak height and peak area were prepared by utilizing the respective Kubelka Munk spectrum obtained for the minimum and maximum vanadate concentration range, in the concentration ratio 1 : 50, of the equivalent amount of V(V) in the same ratio. This was done by measuring absorbance or peak area value at 500 cm^{-1} of the microdrop extract containing VO_2^+-HDPBA complex, dried over KBr (15 mg) substrate, obtained by SDME process for a series of aqueous solutions containing varying known concentrations of V(V). The software IR Solution converts automatically the reflectance spectrum into the Kubelka Munk spectrum for a smoothing of the baseline – see Figure 3(b).

The peak area is an important parameter for the quantification of vanadate because vanadate concentration is found directly proportionate to the peak area. The full range of concentration (1.0–50 μg V(V)/5 mL aqueous phase) was plotted against the respective peak areas obtained for the calibration curve (CCn 1) ($CV^{5+} = 13.5\ A_{peak} - 4.83$). The peak area data obtained for the Kubelka Munk spectrum were processed by the software Table Curve 2D v5.01.01. The peak area data obtained for the Kubelka Munk spectrum showed linearity for this straight line with correlation coefficient, slope and intercept value for the st. line equation as 0.999, 0.568 and 0.145 respectively. Since the correlation coefficient of peak area *versus* concentration curve is greater than 0.99 and being adequately fit, CCn 1 was used for quantitative analysis of V(V) in actual samples.

3.3 *Interference studies*

In the present work, the study on possible interferences due to presence of various types of ions has been carried out. Such as interferences in micro-extraction process, due to solvents and interferences in the DRS-FTIR determination of dichromate due to solvent effects, and other ionic interferences in the determination.

The boiling point of dichloromethane being 39.8–40.0°C it was completely vaporised when the dichloromethane micro drop extract of VO_2^+-HDPBA complex in KBr matrix was dried in the oven. Hence, there was no interferences observed due to the extracting solvent in the vanadate determination and in fact no IR spectral peaks were found for the dichloromethane.

Although large number of organic ligands such as 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Br-PADAP) [4], 2,4,6-tris(2-pyridyl)-1,3,5-triazine(TPTZ) [45], pyrogallol [46] and 1,5-diphenylcarbazide [47] were reported for LLE of vanadium but their selectivity towards other metal ions, particularly Fe(III) which is also an important constituent in biological fluids, is questionable. The HDPBA was a strong and principal component in the micro-extraction process because it was used as selective complexing agent for V(V). Therefore it was necessary to study the interferences of the HDPBA in the determination. We have compared the scanned FTIR spectra of the inorganic vanadate sample and its HDPBA complex in dichloromethane in KBr substrate and found a slight shift (2–5 cm^{-1}) in the vanadate peak positions which is possibly due to coordination of the metal ion with HDPBA in the VO_2^+-HDPBA complex. The organic ligand has appreciable absorbance (~0.05) in blank sample at the working spectral region around 500 cm^{-1}. Therefore, in practice, spectral scans of all SDME sample extracts were measured against a reagent blank. The IR peak for vanadate could be easily identified

in the spectra scanned of vanadate as well as in the spectra of VO_2^+-HDPBA complex, Figure 4(a).

To see the effect of foreign species which are normally found associated with V(V) in real sample, vanadium was determined in model solutions with average content of component ions near to real samples. To study the inter-ionic effect on the change in the intensity ($> \pm 4\%$) and position ($> \pm 3\,cm^{-1}$) of the spectral band at $500\,cm^{-1}$ for standard vanadate at a level of 10 μg V(V) per 5 μL micro drop, varied known concentrations of a large number of inorganic and organic multi-atomic anionic and cationic chemical species were introduced in the analyte solution prior to the SDME process. The band position and spectral intensity of vanadate remained unchanged even in the presence of at least 80-fold molar excess of the following tested multi-atomic cationic and anionic species including, BO_3^{3-}, PO_4^{3-}, AsO_4^{3-}, CO_3^{2-}, SeO_3^{2-}, AsO_3^{2-}, MoO_4^{2-}, FeO_4^{2-}, SiO_4^{2-}, CN^-, OH^-, SCN^-, NO_2^-, ClO_2^-, NO_3^-, ClO_3^-, ClO_4^-, BrO_3^-, IO_3^-, IO_4^-, HCO_3^-, MnO_4^-, NH_4^+, formate, acetate, oxalate, succinate, cinnamate and citrate. Mono-atomic anions and cations like, Cl^-, Br^-, I^-, F^-, Na^+, K^+, Ca_2^+, Mg_2^{2+}, etc. have absolutely no diverse effect.

3.4 *LOD, LOQ and statistical parameters*

The limit of detection (LOD) [48] (defined as the concentration at which we can decide whether an element is present or not, i.e. the point where we can just distinguish a signal from the background concentration of the analyte, or the amount of analyte causing more absorbance than thrice the standard deviation value for 10 replicate measurements of absorbance for blank at 90% confidence limit), of the present method is 40 μg V(V) L^{-1}. The quantitation is generally agreed to begin at a concentration equal to 10 standard deviations of the blank, called the limit of quantitation (LOQ) [48], which is numerically 3.3 times that of the LOD. The LOQ of the present method, thus, is calculated to be 130 μg V(V) L^{-1}. The standard deviation value and the relative standard value at a level of 10 μg V(V)/5 mL aqueous phase for $n = 10$ is found to be 0.26 μg V(V) and 2.6%, respectively.

3.5 *Application of the method*

The proposed method was applied to some natural waters viz. river, ground and rain; and tap and industrial waste water samples collected in and around Raipur, India. Vanadium could then be determined in untreated and treated samples, with DRS-FTIR in KBr matrix after SDME. A small number of real biological samples viz. human blood serum, cerebrospinal fluid and urine were also tested for vanadium content.

Figure 4(b) shows the DRS-FTIR spectra of VO_2^+-HDPBA complex extracted by SDME process from a real human blood serum sample. Table 2 shows the results of analysis of some blood, cerebrospinal fluid and urine samples for their vanadium(V) contents.

To validate the present method, the vanadium(V) content in the tested real samples was also determined by the ICP-MS (PerkinElmer, Elan DRC-e model) technique [49] with flow rate ($L\,min^{-1}$) of 0.9, 1.1 and 15 for nebuliser, auxiliary and plasma gas 501 respectively as standard operating conditions. The results obtained for the same samples by the above two methods were then compared. A high degree of closeness of the analytical data was obtained among the two methods with R.S.D. values ranging between

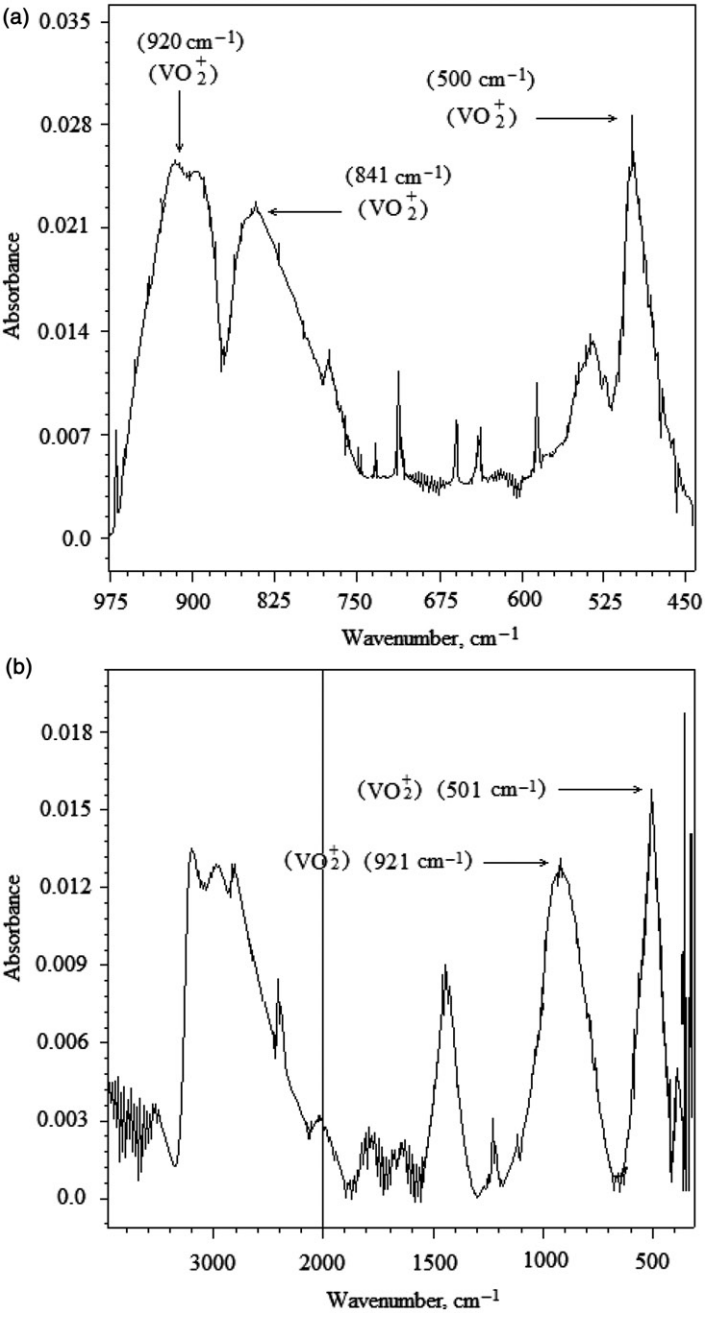

Figure 4. DRS-FTIR spectra of SDME extracted VO_2^+-HDPBA complex in KBr matrix without baseline correction, (a) for standard solution ($^{C}v(v) = 10\,\mu g/5\,mL$), (b) for real water sample, against a reagent blank.

Table 2. Analysis of vanadium by SDME-DRS-FTIR technique in some natural and industrial waste water, and biological samples.

	Amount of vanadium(V) found ($\mu g\,L^{-1}$)					
	Present method		DRC-ICP-MS method [50]			
Sample	V(V)	RSD %, ($n=6$)	V(V)	RSD %, ($n=4$)	F-value[f] (sd_1^2/sd_2^2, $sd_1 > sd_2$)	t-value[g] $\pm t = (\bar{X} - \mu)\frac{\sqrt{N}}{s}$
Water samples						
a	13.0	4.8	13.6	5.3	1.33	2.346
b	56.7	4.9	59.5	5.4	1.34	2.459
c	83.3	4.6	87.3	5.9	1.81	2.547
d	12.2	4.8	12.8	5.8	1.61	2.502
e	1.8	5.6	1.9	3.8	1.93	2.440
Blood serum[#]						
1	2.2	5.6	2.1	5.3	1.25	1.983
2	1.9	6.2	2.0	3.5	2.65	2.085
Cerebrospinal fluid[#]						
1	2.3	7.8	2.4	7.9	1.09	1.363
2	1.9	6.9	2.0	7.3	1.24	2.558
Urine[#]						
1	1.9	5.6	2.0	5.8	1.18	2.499
2	2.0	5.9	2.1	6.7	1.46	2.067

a – Kharun river water, Kumhari, C.G., India.
b – Ground water, Industrial area, Raipur, India.
c – Industrial waste water, Industrial area, Raipur, India.
d – Rain water, Raipur, India.
e – Tap water, Pt. R.S. University, Raipur, India.
f – Tabulated F-values at 95% confidence level is 5.41 for $N_1 = 6$, $N_2 = 4$ (or $\upsilon_1 = N_1 - 1 = 5$, $\upsilon_2 = N_2 - 1 = 3$).
g – Tabulated t-values at 95% confidence level is 2.571 for $N_1 = 6$ (i.e., for $\upsilon_1 = N_1 - 1$, 5 degrees of freedom).
– Biological samples were of patients suffering from different ailments obtained from Pt. J.N. Medical College, Raipur, India.

4.6–7.8% and 3.5–7.9% for the present method and the earlier reported method [50], respectively.

3.6 *Analytical quality assurance test and significance of the method*

In the present work, the F-test was performed at 95% probability to compare the result of the present method with that of the earlier reported method. Because in all cases the values of $F\,(sd_1^2/sd_2^2)$ were less than the tabulated F-values at 95% confidence level the difference between the results of present method and that of the earlier method are not significant (Table 2). Similarly, the t-test was done at 95% confidence. Again, in all cases, the calculated t-values were less than the tabulated values of t, indicating no statistical difference between the results obtained by the two methods (Table 2).

The suitability of an analytical technique is based on several factors such as ease of operation, i.e. the minimum need of technical skill and knowhow, minimal interferences due to sample matrix, the involvement of lesser procedural steps and accessories,

Table 3. Comparison of characteristic features of some of the selected techniques used for determination of vanadium.

Methods	Detection limit ($\mu g\,L^{-1}$)	Analysis time (min)	Sample volume (μL)	Interferences	References
Liquid-liquid extraction Spectrophotometry (LLSE)[a]	5.5	7–10	12,000	Zn^{2+}, Co^{2+} and Fe^{2+}	[4]
Solid-phase extraction – Electrothermal atomic absorption spectrometry (SPE-ETAAS)[a]	0.02	30	20	Na^{+}, Mg^{2+}, Ca^{2+} and silicate ion	[6]
Graphite furnace atomic absorption spectrometry (GF-AAS)[a]	2.6–7.0	20–60	5–200 mL	Na^{+}, Mg^{2+}, Ca^{2+}, Cl^{-}, Cu^{2+}, Zn^{2+}, Sr^{2+}, Fe^{3+}, As^{5+}, Al^{3+}, Co^{2+}, Hg^{2+}, NO_3^- and SO_4^{2-}	[9]
Flow injection analysis (FIA)[a]	1.98×10^{-7} and 2.97×10^{-7} mol dm^{-3}	5	80	Co^{2+}, Cr^{6+}, Zn^{2+} and Fe^{3+}	[45]
Inductive coupled plasma mass spectrometry (ICP-MS)[a]	5.5	5	20	$^{40}Ar^{12}C^{+}$ and $^{40}Ar^{13}C^{+}$	[49]
Dynamic Reaction Cell-Inductive coupled plasma mass spectrometry (DRC-ICP-MS)[a]	12–30 ng L^{-1}	10	10–20	$^{40}Ar^{35}Cl^{+}$ and $^{35}Cl^{+16}O$	[50]
Single-drop microextraction-DRS-FTIR (SDME-DRS-FTIR)[b]	40	3	5	No interference	Present method

a – Large quantities and a number of consumables (chemicals, solvents, gases etc.) are required.
b – Micro amounts and small number of consumables are required.

possibility to avoid dealing with large number and volume of reagents and chemicals in view to the current trend of green-chemistry work and of course the detection and quantification limits. The liquid-liquid extraction spectrophotometry (LLSE) [4], due to operating ease, are though popular method for vanadium determination but these methods deal with huge amount of sample volume. The solid-phase extraction–electrothermal atomic absorption spectrometry (SPE-ETAAS) [6] technique needs costly consumables and matrix interference is also a major concern. Graphite furnace atomic absorption spectrometry (GF-AAS) [9] and flow injection analysis [45] methods also require elimination of foreign ions and requirement of large sample volume limits the applicability of these methods. The ICP-MS techniques [49,50] though are sensitive but involve large technical complexity and also involve removal of interferences of polyatomic ions viz. $^{40}Ar\ ^{12}C^{+}$ and $^{40}Ar^{13}C^{+}$ prior to analysis. The comparison on characteristic features of some of the selected techniques used for the determination of vanadium shows the significance and suitability of the present method (see Table 3).

4. Conclusions

The main difficulty in the determination of vanadium in environmental water samples is its low concentration level. The SDME-DRS-FTIR procedure developed has shown adequate sensitivity and simplicity, as only few drop of chelating reagent used for the extraction of vanadium. The high selectivity of the method for determination of vanadium by DRS-FTIR permits its application for the analysis of different kinds of waters and biological sample. This system of preconcentration associated with DRS-FTIR allowed the determination of vanadium in water and biological samples with good reproducibility and accuracy at concentrations of micro gram. The method is simple and can be easily adapted to any laboratories and is suitable for routine analysis competing with sophisticated hyphenated techniques.

Analytical requirements for the measurement of V in blood, cerebrospinal fluid (CSF) and urine are very stringent in order to ensure a reliable sample and hence accurate data. The analysis of micro components with the limitations of small available sample size, e.g. in case of cerebrospinal fluid, is a real challenge to the analytical chemist. The newly developed hyphenated technique SDME-DRS-FTIR could be efficiently employed in such cases where available sample size is very small. The present method requires no large amount of consumables and hence is also cost effective.

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References

[1] R.J. French and P.J. Jones, Life. Sci. **52**, 339 (1993).
[2] M. Almeida, S. Filipe, M. Humanes, M.F. Maia, R. Melo, N. Severino, J.A.L. da Silva, J.J.R. Fraústo da Silva, and R. Wever, Phytochem. **57**, 633 (2001).

[3] R. Liasko, T.A. Kabanos, S. Karkabounas, M. Malamas, A.J. Tasiopoulas, D. Stefanou, P. Collery, and A. Evangelou, Anticancer Res. **18**, 3609 (1998).
[4] A.C.S. Costa, S.G.L. Teixeira, H.V. Jaeger, and S.L.C. Ferreira, Mikrochim. Acta **130**, 41 (1998).
[5] M.K. Deb, K.S. Patel, and R.K. Mishra, Intern. J. Environ. Anal. Chem. **43**, 209 (1991).
[6] I. Nukatsuka, Y. Shimizu, and K. Onzeki, Anal. Sci. **18**, 1009 (2002).
[7] Q. Hu, G. Yang, Z. Huang, and J. Yin, Bull. Korean Chem. Soc. **25**, 263 (2004).
[8] F. Aureli, S. Ciardullo, M. Pagano, A. Raggi, and F. Cubadda, J. Anal. At. Spectrom **23**, 1009 (2008).
[9] X. Zhu, Z. Zhu, and S. Wu, Microchim. Acta **161**, 143 (2008).
[10] Z. Fan, B. Hu, and Z. Ijang, Spectrochim. Acta Part B **60**, 65 (2005).
[11] M. Takaya, Ind. Health **38**, 91 (2000).
[12] L. Li and B. Hu, Talanta **72**, 472 (2007).
[13] M. Gharehbaghi, F. Shemirani, and M. Baghdadi, Intern. J. Environ. Anal. Chem. **89**, 21 (2009).
[14] M.J. Jung, Y.J. Shin, S.Y. Oh, N.S. Kim, K. Kim, and D.S. Lee, Bull. Korean Chem. Soc. **27**, 231 (2006).
[15] D.C. Wood, J.M. Miller, and I. Christ, LCGC North America **22**, 516 (2004).
[16] R. Zhao, W. Lao, and X. Xu, Talanta **62**, 751 (2004).
[17] J.M. Kokosa and A.J. Przyjazny, Chromatogr. A **983**, 205 (2003).
[18] X. Li, X. Xu, X. Wang, and L. Ma, Intern. J. Environ. Anal. Chem. **84**, 633 (2004).
[19] W. Liu and H.K. Lee, Anal. Chem. **72**, 4462 (2000).
[20] Y. He and H.K. Lee, J. Chromatogr. A **1122**, 7 (2007).
[21] A.D. Guimaraes, J.J. Carvaiho, C. Goncalves, and M.F. Alpendurada, Intern. J. Environ. Anal. Chem. **88**, 151 (2008).
[22] L. Zhu, L. Zhu, and H.K. Lee, J. Chromatogr. A **924**, 407 (2001).
[23] Y. He and Y.J. Kang, J. Chromatogr. A **1133**, 35 (2006).
[24] X. Jiang and H.K. Lee, Anal. Chem. **76**, 5591 (2004).
[25] D. Verma, S.K. Verma, and M.K. Deb, Talanta **78**, 270 (2009).
[26] G. Wei, Y. Li, and X. Wangy, Intern. J. Environ. Anal. Chem. **88**, 392 (2008).
[27] E. Carasek, J.K. Tonjes, and M. Scharf, Talanta **56**, 185 (2002).
[28] M. Gallignani, M. Valero, C. Ayala, M. del Rosario Brunetto, A. Sánchez, J. Luis Burguera, and M. Burguera, Talanta **64**, 1290 (2004).
[29] K.P. Ska and T. Wierzbicki, Talanta **64**, 823 (2004).
[30] F.A.C. Amorim, B. Welz, A.C.S. Costa, F.G. Lepri, M.G.R. Vale, and S.L.C. Ferreira, Talanta **72**, 349 (2007).
[31] Z.L. Chen and G. Owens, Anal. Chim. Acta **607**, 1 (2008).
[32] L. Zhao, X. Zhu, K. Feng, and B. Wang, Intern. J. Environ. Anal. Chem. **86**, 931 (2006).
[33] S.K. Verma and M.K. Deb, J. Agric. Food Chem. **55**, 8319 (2007).
[34] S.K. Verma and M.K. Deb, Talanta **71**, 1546 (2007).
[35] S.K. Verma, M.K. Deb, and D. Verma, Atmos. Res. **90**, 33 (2008).
[36] S.K. Verma and M.K. Deb, Anal. Chim. Acta **582**, 382 (2007).
[37] E.B. Sandell, *Colorimetric Determination of Traces of Metals* (Interscience, New York, 1959).
[38] K. Satyanarayana and R.K. Mishra, Anal. Chem. **46**, 1609 (1974).
[39] G. Schlemmer and B. Welz, Spectrochim. Acta, Part B **41**, 1157 (1986).
[40] O. Acar, Anal. Sci. **22**, 731 (2006).
[41] L.G. Sillen, Acta Chem. Scand. **10**, 185 (1956).
[42] F.F. Canwell and M. Losier, in *Wilson & Wilson's Comprehensive Analytical Chemistry*, edited by J. Pawliszyn and D. Barcelo (Elsevier, Amsterdam, 2002), p. 297.
[43] L. Zhao and H.K. Lee, J. Chromatogr. A **919**, 381 (2001).
[44] R.A. Nyquist and R.O. Kagel, *Infrared Spectra of Inorganic Compounds* (Academic Press, New York, 1971).

[45] J. Wei, N. Teshima, and T. Sakai, Anal. Sci. **24**, 371 (2008).
[46] N. Iranpoor, N. Maleki, S. Razi, and A. Safavi, Talanta **39**, 281 (1992).
[47] M.J. Ahmed and S. Banoo, Talanta **48**, 1085 (1999).
[48] V. Thomsen, D. Schatzlein, and D. Mercuro, Spectroscopy **18**, 112 (2003).
[49] Z.L. Chen, M.M. Rahman, and R. Naidu, J. Anal. At. Spectrom. **22**, 811 (2007).
[50] V. Chrastny, M. Komarek, M. Mihaljevic, and J. Stichova, Anal. Bioanal. Chem. **385**, 962 (2006).